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Assessment of dominance hierarchy through urine scent marking and its chemical constituents in male blackbuck *Antelope cervicapra*, a critically endangered species

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ABSTRACT

In ungulates the process of chemical communication by urinary scent marking has been directly related to reproductive dominance, territorial defense and proximity to resources. The differences in the frequency of urine marking and chemical composition of urine of males *Antelope cervicapra* before, during and after the dominance hierarchy period were assessed. The variations in the urine marking and its chemical profiles of dominant males ($n = 9$), bachelors ($n = 5$) and sub-adult males ($n = 5$) were compared to find out how the dominance hierarchy influences the confined blackbuck herd under semi-natural captive conditions. The frequency of urine marking is significantly higher ($p < 0.001$) in dominant males. Twenty-eight major constituents were identified in the urine of dominant males (before, during and after the dominance hierarchy period), bachelor and sub-adult males. Among these, three specific compounds namely, 3-hexanone (I), 6-methyl-5-hepten-2-one (II) and 4-methyl-3-heptanone (III) were seen only in dominant males urine during the dominance hierarchy period. Based on the behavioural observation and the unique chemical constituents in the urine, it is concluded that the dominant male scent odor suppresses aggression, scent marking, scent production and territorial patrolling activities of subordinate males, through which the dominant male establish their hierarchy and attains success in reproduction.

1. Introduction

Many ungulates are socially characterized by well-defined stable dominance hierarchies (Cassinello, 1995; Cote, 2000; Freeman et al., 2004; Roden et al., 2005) which often determines the first or the best access to food, social interactions, and choice of mate (Roden et al., 2005; Hemelrijik et al., 2008). The hierarchical position of an individual is influenced by various factors including age (*Bison bison*: Maher and Byers, 1987; Robitaille and Prescott, 1993), body weight (*Gazella dama*: Cassinello and Pieters, 2000), both age and body weight (*Bison bison*: Roden et al., 2005), aggressiveness (*Oreamnos americanus*: Cote, 2000; *Capra hircus*: Barroso et al., 2000; *Loxodonta africana*: Ganswindt et al., 2005), androgen level (*Pupu puda*: Barto et al., 1998; *Elaphurus davidianus*: Li et al., 2004; *Pan troglodytes*: Muller and Wrangham, 2004) and dominance hierarchy is characterized by scent marking/production (*Lemur catta*: Kappeler, 1990; *Mus domesticus*: Hurst, 1990; *Oreotragus oreotragus*: Roberts and Dunbar, 2000; *Propithecus uerreauxi uerreauxi*: Lewis, 2005; *Equus Caballus*: Kimura, 2001; *Meriones unguiculatus*: Shimozuru et al., 2006).

Typically urinary scent marking involves deposition of social pheromones to elicit response from a conspecific (Ewer, 1968; Bowyer et al., 1994; Hoffman et al., 2010). Scent marking behaviours of many ungulates have been described (Gosling, 1985; Bowyer et al., 1994); urine and scent glands are the major sources of the odors (Roberts and Dunbar, 2000; Gosling and Roberts, 2001; Lewis, 2005). Major functions of urinary scent marking are defense of territory and resources, advertisement of social status, regulation of social relationships, mate attraction, and advertisement of reproductive condition (Halpin, 1986; Penn and Potts, 1998; Smith et al., 2001; Brennan and Kendrick, 2006).

In many ungulates urinary scent marking behaviour not only mediates aggressive interactions between males but also facilitates male–female interactions. For example, odors of urinary scent marking help to establish the territory of dominant male and also to keep the other males away from its territory (Thomsons's gazelles, *Gazella Thomson*: Estes, 1967; Blackbuck, *Antelope cervicapra*: David, 1973). In ungulates like bontebok, *Damaliscus dorcas dorcas* (Schaller, 1967), springbok, *Antidorces marsupialis* (David, 1973) and wildebeest, *Connochaetes taurinus* (Estes, 1969) urinary

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scent marking directs other males as part of a ritualized challenge. Urinary scent marking in North American elk, *Cervus elaphus* primarily influences the dominance interactions between adult males and the urinary pheromones might advertise the physical condition of males (McCullough, 1969; Bowyer and Kitchen, 1987). Male urine excreted during rut has a strong, pungent and unique odor which relays information an dominance hierarchy to conspecifics (Miquelle, 1991) indicating the urine of males may possess critically important components required in establishing territory and/to attract females.

Urinary constituents of a few ungulates have been characterized, but the biological role of urinary scent marking is yet to be investigated. For example, the red deer urine consisted mainly that derivative of carboxylic acids and some aromatic compounds (Bakke and Figenschou, 1990). Volatile substances identified in the urine of white-tailed deer comprise alcohol, aldehyde, furan, ketone, nitrite, alkene, alkane, thiol ester, disulfide, aromatic ether, ketal and amine classes of compounds (Miller et al., 1998). The preselection of candidate substances has further resulted in successful characterization of a few biological urinary pheromones in various zoo animals, farm animals and rodents. For example, urine of Asian female elephants in estrus contain high concentration of a volatile (Z)-7-dodecen-1-yl acetate compound which function as a sex pheromone stimulating male sexual behaviour (Rasmussen et al., 1997). In addition, the estrus-specific urinary volatile, 1-iodoundecane may function as bull attractant (Rameshkumar and Archunan, 2002). The male mice urinary pheromones like 2-sec-butyl-dihydrothiazole and dehydro-exo-brevicomin were active in eliciting inter-male aggression (Novotny et al., 1985a), attractiveness to females (Jemiolo et al., 1985), and estrus synchronization (Jemiolo et al., 1986). Another urinary volatile compound 2,5-dimethylpyrazine delivered from grouped adult females, delayed sexual maturation among young female mice (Novotny et al., 1985b). The urinary compounds and putative pheromones of dominant and subordinate male blackbucks are yet to be identified.

The Indian Blackbuck, *A. cervicapra* is territorial and generally lives in herds 30–100 individuals. The regulation of its social life depends to a large extent on chemical communication as reflected by its various odoriferous skin glands and other sources of chemical signals. Each group has a territory within which a linear dominance hierarchy is formed by means of aggression interactions. Previous study has demonstrated that the Indian Blackbucks scent mark their territory with urine and preorbital glands (David, 1973; Manimozhi, 1996; Rajagopal and Archunan, 2008; Rajagopal et al., 2010). We tested the hypothesis that there were differences in the frequency of scent urination and its chemical composition in dominant male before, during and after the dominance hierarchy period when compared with that of bachelor male. The study is a first step in understanding the potential role of urine marking in the dominance hierarchy of Indian Blackbuck.

2. Material and methods

2.1. Study area

This study was conducted in the conservation and breeding centre of Arignar Anna Zoological Park (AAZP) (13°16′S and 79°54′E at an altitude of MSL+ 10–100 m), Vandalur, Chennai, South India. Chennai has the distinction of being the first zoo in India, started in 1855. In 1976, the zoo was moved to the Vandalur Reserve Forest comprising, an area of about 510 ha near Chennai. The habitat of AAZP is considered a tropical evergreen scrub, a degraded forest mostly consisting of thorny bushes. The average annual rainfall is about 250 mm and the temperature about 26 °C.

Table 1
Antelope cervicapra L.: identification features of adult male blackbucks.

Animal (No.)	Number of twists in horn	Identification characters
1st	4[a]	Narrow horns with pointed tips; A large size mole in the lower abdomen on left side
2nd	3[a]	Almost parallel horns with pointed tips
3rd	3[a]	Left horn normal; right horn damaged and half bent
4th	4[a]	Slightly twisted narrow horns with curved tips pointing each other
5th	3[a]	Broad and compressed horn; tip of the right horn curved outward
6th	3[a]	Long broad horns with curved tips outward
7th	3[a]	Broad horns with their tips curved inward
8th	3[a]	Long narrow horns with their tips curved inward
9th	3[a]	Long broad horn; tip of the right horn damaged
10th	2[a]	Narrow twisted horns, tip of the both horns very long and pointed
11th	3[a]	Narrow horns, tip of the left horn little damaged
12th	3[b]	Long twisted slim horn, tip of the right horn curved inward; right horn outward
13th	2[a]	Long parallel horns with their tips straight onward sky
14th	2[a]	Broad horns; a large size scare in the right hind leg

[a] Thick solid horn.
[b] Slim solid horn.

2.2. Study animals

In the beginning of the study period (June 2007) the Blackbuck enclosure contained a total population of 75 animals. The group was classified (Prasad, 1983) into 14 adult males, 21 adult females, 16 sub-adult males, 18 sub-adult females and 6 young ones. The social hierarchy system (i.e. dominance hierarchy) was studied in 14 adult males only. Each individual was recognized by variation in shape of the horn and additional morphological features (Table 1).

2.3. Social status of male blackbuck

The nomenclature of male social status are (Hogg and Forbes, 1997; Mungall, 1977): (1) harem masters (i.e. dominant males) who hold harems, (2) challengers (i.e. predominant males) without their own harems, but challenge the harem master and try to hold females, (3) the bachelor group which stay away from female groups. Indian Blackbucks change their social status from 'bachelor' to 'challenger' and became 'harem masters'; but some of them (bachelors) may never reach the highest social rank during their lifetime (Mungall, 1977).

2.4. Behavioural observation

Frequency of urine marking behaviour was observed in adult males ($n = 14$) using focal sampling method (Altmann, 1974). The observations were made for 122 days during 18 months from June 2007 to December 2008. The observation schedule was divided into two shifts: morning 08.00–10.00 h and afternoon 14.00–16.00 h. The duration of each watch for 2 h each comprised, a total of 4 h of observation per day. The animals were directly observed using 8×40 binoculars in front of the enclosure. The observation included mapping all visible scent marks and recording the type and locations of marks.